

Lorvotuzumab mertansine, a CD56-targeting antibody-drug conjugate with potent antitumor activity against small cell lung cancer in human xenograft models

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Abbreviations: ABC, antibodies bound per cell; ADC, antibody-drug conjugate; chKTI, chimeric anti-soybean trypsin inhibitor antibody; CR, complete tumor regression; DM1, maytansinoid effector molecule; FBS, fetal bovine serum; FFPE, formalin-fixed paraffin-embedded; huN901, lorvotuzumab; IACUC, Institutional Animal Care and Use Committee; IgG, immunoglobulin G; IHC, immunohistochemistry; LCK, log cell kill; LM, lorvotuzumab mertansine; MED, minimal efficacious dose; MTD, maximum tolerated dose; NCAM, neural cell adhesion molecule; NK, natural killer cell; PBS, phosphate buffered saline; PE, phycoerythrin; PR, partial tumor regression; SCID, severe combined immunodeficient; SCLC, small cell lung cancer; SPP, N-succinimidyl-3-(2-pyridyldithio)propionate ; T/C, tumor growth inhibition; T-C, tumor growth delay

Lorvotuzumab mertansine (LM) is an antibody-drug conjugate composed of a humanized anti-CD56 antibody, lorvotuzumab, linked via a cleavable disulfide linker to the tubulin-binding maytansinoid DM1. CD56 is expressed on most small cell lung cancers (SCLC), providing a promising therapeutic target for treatment of this aggressive cancer, which has a poor five-year survival rate of only 5–10%. We performed immunohistochemical staining on SCLC tumor microarrays, which confirmed that CD56 is expressed at high levels on most (~74%) SCLC tumors. Conjugation of lorvotuzumab with DM1 did not alter its specific binding to cells and LM demonstrated potent target-dependent cytotoxicity against CD56-positive SCLC cells in vitro. The anti-tumor activity of LM was evaluated against SCLC xenograft models in mice, both as monotherapy and in combination with platinum/etoposide and paclitaxel/carboplatin. Dose-dependent and antigen-specific anti-tumor activity of LM monotherapy was demonstrated at doses as low as 3 mg/kg. LM was highly active in combination with standard-of-care platinum/etoposide therapies, even in relatively resistant xenograft models. LM demonstrated outstanding anti-tumor activity in combination with carboplatin/etoposide, with superior activity over chemotherapy alone when LM was used in combinations at significantly reduced doses (6-fold below the minimally efficacious dose for LM monotherapy). The combination of LM with carboplatin/paclitaxel was also highly active. This study provides the rationale for clinical evaluation of LM as a promising novel targeted therapy for SCLC, both as monotherapy and in combination with chemotherapy.

Introduction

Small cell lung cancer (SCLC) accounts for approximately 13% of lung cancer cases, which amounted to approximately 33,000 new cases in the United States in 2010, and approximately 170,000 new cases worldwide.^{1–3} Approximately 40% of newly diagnosed patients with SCLC have limited-stage disease where the disease is confined to the hemithorax, while the majority of patients (57%) have extensive-stage SCLC at diagnosis with disease presenting beyond that region.^{3,4} Patients with both limited-stage and extensive-stage disease respond well to

standard-of-care cisplatin/etoposide therapy with response rates ranging from 60–90%. However, despite the initial responses to chemotherapy, the prognosis for SCLC is poor; median survival is about 18 mo in limited-stage disease with 5-y survival rates of 12%, and only 10–12 mo in extensive-stage SCLC with 2-y survival of less than 5%.^{3–5} For limited-stage SCLC, treatment with cisplatin/etoposide and concurrent thoracic radiotherapy is now considered standard-of-care, having shown increased overall survival vs. chemotherapy alone.⁶ Carboplatin/etoposide has been evaluated in various clinical trials and found to be comparable in activity to cisplatin/etoposide therapy, with reduced toxicities of

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emesis, neuropathy and nephrotoxicity, but with an increased risk of myelosuppression.^{7,8} However, for extensive stage SCLC, there have been few advances in treatment beyond platinum/etoposide regimens. Clinical investigations of non-platinum containing regimens or platinum/etoposide triplet combinations with various agents, including irinotecan, topotecan, gemcitabine, paclitaxel and pemetrexed have been unsuccessful in improving response rates or overall survival, and some have been accompanied by increased toxicity.⁸⁻¹⁰ Single-agent topotecan is the only agent approved for relapsed, platinum-sensitive SCLC in patients after failure of first-line therapy with median progression-free survival of only 13.3 wk.¹⁰ Thus, despite the efforts to improve patient outcome, the current state of SCLC treatment remains inadequate, and there is a need for therapeutics with increased activity and more durable responses.

Lorvotuzumab mertansine (LM, huN901-SPP-DM1, BB-10901, IMGN901) is an antibody-drug conjugate (ADC) composed of the humanized anti-CD56 antibody lorvotuzumab (huN901) linked to the cytotoxic maytansinoid effector molecule DM1 via a disulfide linkage.¹¹⁻¹³ N901 is a murine IgG1 monoclonal antibody that binds human CD56, a member of the neural cell adhesion molecule (NCAM) family.¹⁴ CD56 is expressed on natural killer (NK) cells and CD56-positive T lymphocytes, but not expressed on monocytes, B-lymphocytes, most T-lymphocytes, erythrocytes or platelets.¹⁴ The N901 antibody also binds to CD56 expressed by tumor cells in a majority of SCLC tumor cases, as well as on cells of other tumors of neuroendocrine origin.¹⁵⁻²⁰ CD56 is also expressed on approximately 88% of Merkel cell cancers (22 of 25 patients), more than 70% of multiple myeloma cases, as well as on many cases of ovarian cancer.²¹⁻²⁵

LM has been shown to have potent activity against CD56-positive multiple myeloma cell lines in vitro and xenografts in vivo, and is currently under clinical investigation in combination with lenalidomide/dexamethasone in patients with multiple myeloma.²⁵ Herein, we describe for the first time in a peer-reviewed journal, the results of preclinical studies in SCLC which, along with the high level of CD56 expression in SCLC, provided the rationale for clinical evaluation of LM as monotherapy and in combination with chemotherapy in SCLC.

Results

CD56 expression in SCLC tumors

Detection of CD56 in SCLC tumors using immunohistochemistry (IHC) has been described, with up to 100% of SCLC cases found to be positive for CD56 expression.²⁶⁻²⁸ To expand upon these findings, we performed CD56 IHC on a panel of formalin-fixed paraffin-embedded (FFPE) SCLC tumor microarrays (117 samples total), using a commercially-available anti-CD56 IHC antibody. Under staining conditions that were optimized to allow estimation of CD56 expression level based on calibrated control cell pellets, a total of 93% (109 of 117) of the SCLC samples were positive for CD56. All samples were evaluated by a board-certified pathologist to confirm the anatomic site, tumor type, and integrity of the tissue prior to scoring. Samples

of poor quality (e.g., necrotic, crushed) and tumor samples that did not contain invasive tumor were not evaluated further. The distribution of IHC scores of the samples is represented in **Figure 1A**; the shaded subset of samples scored with intensity level 2 or greater ($\geq 2+$) with a heterogeneous or homogeneous distribution pattern clearly localized to the tumor cell plasma membrane are considered to have high CD56 expression levels (74%, 87 of 117). This group of samples is estimated to have a minimum of 160,000 CD56 antigens expressed per cell, based on the cell pellet IHC scores. Representative images of SCLC samples with varying levels of staining relative to calibrated cell pellets are shown in **Figure 1B**.

Targeting CD56 with LM

The N901 antibody was humanized as described by Roguska et al.^{14,29} This humanized antibody, lorvotuzumab, was conjugated to DM1 using the SPP linker to form LM with an average of 3.5 molecules of DM1 linked per antibody as described in Materials and Methods.¹¹ Binding of LM to the human SCLC line, SW2, was shown to be comparable to the binding of unmodified lorvotuzumab and the murine N901 “parent” antibody, with high apparent binding affinity EC_{50} values of 0.4, 0.3, and 0.2 nM, respectively (**Fig. 2A**). Similar binding of lorvotuzumab and LM was demonstrated against other CD56-expressing cells, including multiple myeloma and ovarian cancer cell lines (data not shown). Thus, the high-affinity, antigen-specific binding of lorvotuzumab is comparable to the murine N901 antibody and is not appreciably affected by conjugation with the maytansinoid cytotoxic agent.

To investigate the CD56-targeted potency of LM, we performed in vitro assays to assess cell killing using two CD56-expressing SCLC cell lines: NCI-H526 and NCI-H69. As shown in **Figure 2B**, LM demonstrates targeted cell-killing activity in vitro against both cell lines, with IC_{50} values of 0.2 nM for NCI-H526 and 5 nM for NCI-H69. The antigen-dependency of this activity is confirmed by the absence of cell killing in the presence of excess unconjugated lorvotuzumab (antibody binding alone has no effect on cell growth).

Anti-tumor activity of LM in xenograft models of SCLC

Dose-dependent anti-tumor efficacy of LM was demonstrated in the SW2 SCLC xenograft model using single-dose IV administration of 3, 17, and 51 mg/kg (**Fig. 3A**; **Table 1A**). The minimal efficacious dose (MED) of LM was 3 mg/kg (T/C = 29%). High activity (T/C < 10%) was observed at the dose of 17 mg/kg, with half of the animals tumor-free at the end of the study. The 51 mg/kg dose was curative; all animals had complete tumor regressions and remained tumor-free for the duration of the study. The tolerability of the treatments was assessed by regular monitoring of clinical signs and body weight. Body weight loss was observed in animals in the 51 mg/kg treatment group only (11% mean body weight loss at nadir, day 4 post treatment) suggesting that this dose was near the maximum tolerated dose (MTD) in the immune-deficient SCID mice. No signs of toxicity were observed at the two lower doses. In a separate experiment, the MTD of LM in CD-1 mice was approximately 90 mg/kg (data not shown). As lorvotuzumab does not bind to murine CD56, the MTD in CD-1 mice represents non-targeted,

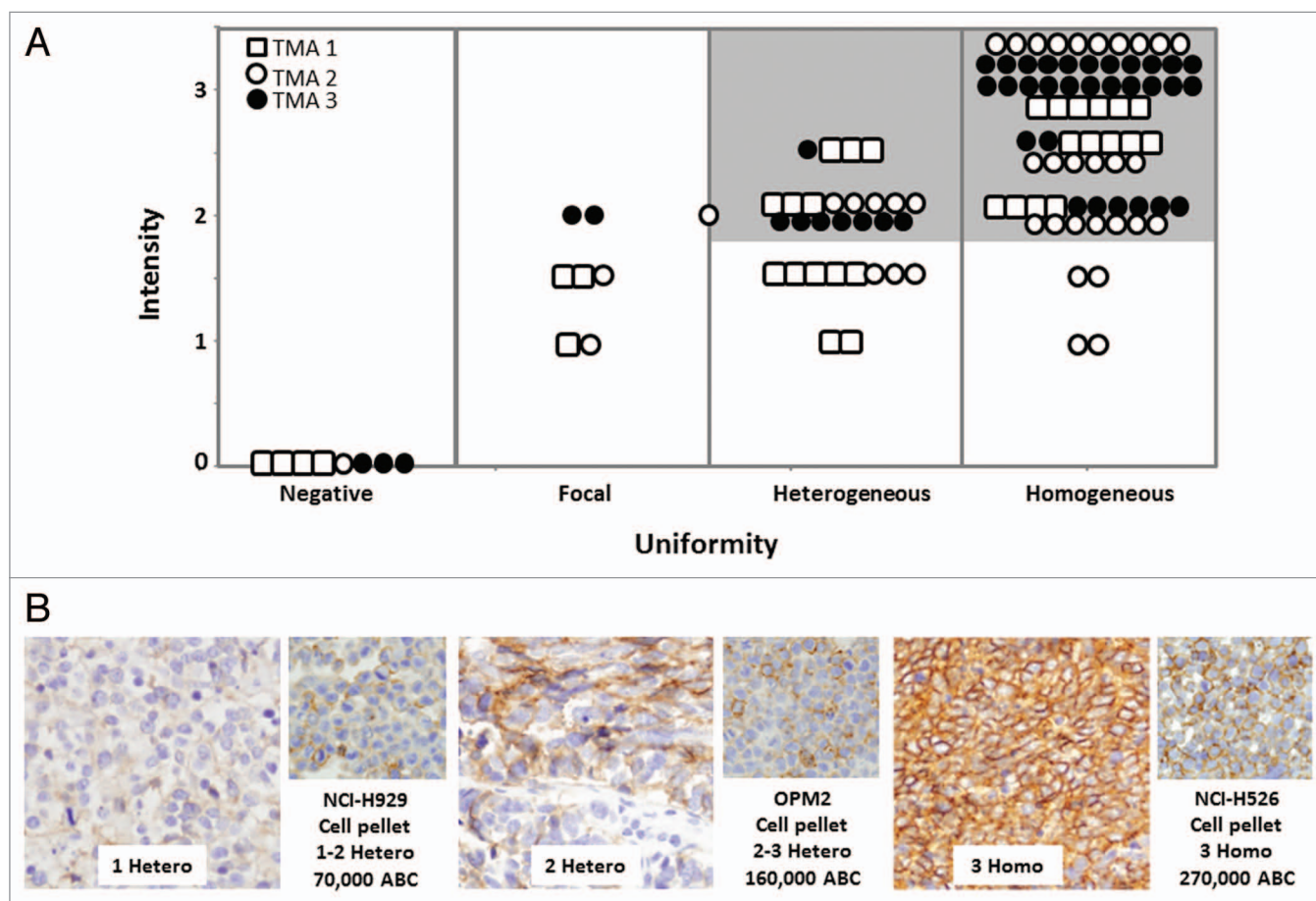


Figure 1. (A) Distribution of CD56 staining levels in the three SCLC tumor microarrays. IHC scores of all TMA sections demonstrate that a total of 93% (109 of 117) of the SCLC samples were positive for CD56; the shaded subset of samples scored at an intensity level 2 or greater (≥ 2) are considered high CD56 expression levels (74%, 87 of 117). **(B)** Representative examples of staining scores for TMA samples relative to CD56 ABC calibrated cell pellet controls. FFPE cell pellets of NCI-H526 SCLC, OPM2 multiple myeloma (MM) and NCI-H929 MM were stained as a calibrated CD56 expression level reference. CD56 expression levels on cells at the time of fixation and paraffin embedding was determined by flow cytometry, quantified as antibodies bound per cell (ABC) for each test sample by using mean fluorescence value of bound lorvotuzumab-PE and a calibration curve generated with BD Quantibrite™ beads.

antigen-independent tolerability. The effect of LM on normal tissues expressing CD56 cannot be assessed in mice.

Conjugation of lorvotuzumab with a potent cell-killing agent such as DM1 is required for antitumor activity: treatment of mice bearing NCI-H526 xenografts with unconjugated lorvotuzumab (8.5 mg/kg weekly \times 2) and free maytansinoid (DM1-SMe, 150 μ g/kg weekly \times 2) was not effective (Fig. 3B; Table 1B). DM1-SMe is a mixed disulfide of DM1 with thiomethane, which blocks the free thiol of DM1, resulting in a free DM1 form most representative of conjugated DM1. A non-targeting conjugate control, chKTI-SPP-DM1 was also inactive in this model, whereas LM was active at the matched dose and schedule (8.5 mg/kg Ab/150 μ g/kg DM1 as conjugated ADC weekly \times 2), causing complete tumor regressions in 2 of 6 mice, demonstrating the antigen-specificity of LM (Fig. 3B; Table 1B).

In vivo activity of LM in combination with chemotherapeutics

Based on the emerging clinical experience on the tolerability of maytansinoid-ADCs and considering that most cancer therapies are used typically in combination with other chemotherapeutics,

we investigated the activity of LM in combination with other agents.^{30,31}

LM was evaluated in combination with platinum/etoposide, which is standard-of-care therapy for SCLC, against two SCLC xenograft models that varied in their sensitivity to LM and to platinum/etoposide chemotherapy alone.⁵ LM demonstrated dose-dependent activity against NCI-H526 xenografts (Fig. 4A): a dose of 8.5 mg/kg (qw \times 2) was active and 17 mg/kg (qw \times 2) was highly active, with 4 of 6 animals tumor-free at the end of the study. Carboplatin and etoposide were inactive as monotherapy and had a minimal level of activity in this model when used in combination (Fig. 4A). In a separate experiment, mice bearing NCI-H526 xenografts were treated with LM (8.5 mg/kg qw \times 2) in combination with carboplatin, etoposide or carboplatin/etoposide (Fig. 4B). Combination of LM with carboplatin/etoposide was highly active, resulting in durable complete tumor regressions in 5 of 6 animals. Combination with carboplatin alone was also highly active, whereas combination with etoposide alone was comparable to LM monotherapy.

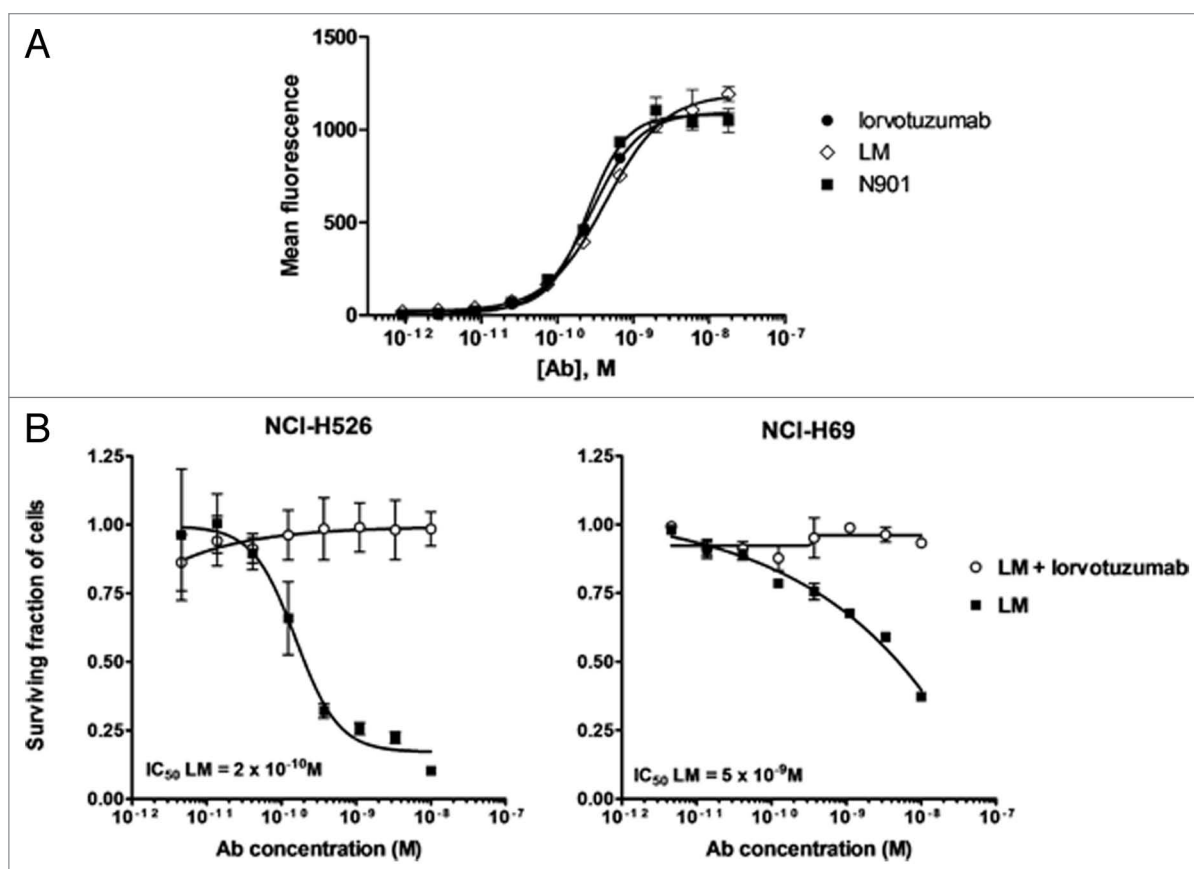


Figure 2. (A) Binding of N901, lorvotuzumab and LM to SW2 SCLC cells. Cells were incubated with serial dilutions of N901, lorvotuzumab, or LM as described in Materials and Methods. Bound antibody or conjugate was detected with fluorescein-labeled anti-mouse or anti-human IgG. Cells were assessed on a FACScan flow cytometer and results were analyzed by CellQuest Pro. Mean fluorescence (\pm SD) is shown for each concentration. (B) In vitro activity of LM against SCLC cell lines: NCI-H526 and NCI-H69. Cells were treated with serial dilutions of LM with or without excess (1 μ M) lorvotuzumab (blocking Ab) at 37 °C for 8–9 d, and viability was assessed utilizing a WST-8 cell viability assay. The fraction of surviving cells is shown for each concentration of LM. (1 nM = 146 ng/ml antibody concentration; 3.5 nM DM1 = 2.6 ng/ml based on a molar ratio of 3.5 DM1 molecules linked per antibody molecule).

Results of LM monotherapy and chemotherapy regimens are summarized in Table 2A, with combination data in Table 2B.

Treatment with LM in combination with platinum/etoposide was also evaluated against NCI-H69 xenografts. NCI-H69 cells were about 20-fold less sensitive to treatment with LM in vitro than NCI-H526 cells. This lower sensitivity to treatment was also observed in vivo: LM monotherapy was inactive in the NCI-H69 xenograft model (T/C > 42%) at the dose evaluated (18 mg/kg qw \times 2) as shown in Figure 4C. NCI-H69 xenografts were also generally insensitive to chemotherapy: carboplatin/etoposide was inactive and cisplatin/etoposide had only modest activity. Despite the inactivity of LM monotherapy, combination with the inactive carboplatin doublet was active, and combination with the modestly active cisplatin doublet was highly active (Fig. 4C; Table 2C). Partial tumor regressions were observed in only 1 of 8 animals treated with LM monotherapy, in 2 of 8 animals in the LM/carboplatin/etoposide group and 4 of 8 animals in the LM/cisplatin/etoposide group. The lack of complete tumor regressions is indicative of the relative resistance of this model to treatment.

To assess the magnitude of combination potency, combinations of carboplatin/etoposide plus reduced-dose LM were

evaluated in the NCI-H526 xenograft model (Fig. 5). Animals were treated with single-agent LM at the MED of 8.5 mg/kg, and at reduced doses of 5.7, 2.8, and 1.4 mg/kg (qw \times 2). The 8.5 mg/kg LM dose was highly active (T/C = 5%), with 3 of 6 mice tumor-free until the end of study (day 111) (Fig. 5A), whereas all reduced-doses of LM were inactive (Fig. 5B–D). Carboplatin/etoposide chemotherapy was active (T/C = 16%), with no tumor-free survivors. As in the previous study, combination of LM (8.5 mg/kg) with carboplatin/etoposide was highly active (T/C = 0%), with 5 of 6 animals remaining tumor-free at day 111. All combinations with dose-reduced LM plus carboplatin/etoposide were also highly active. The 5.7 and 2.8 mg/kg combinations both resulted in T/C values of 0%, with 4 of 6 and 3 of 6 tumor-free survivors, respectively (Fig. 5B and C). Even the combination with the lowest LM dose (representing a 6-fold dose reduction below the MED) resulted in a T/C of 5%, with 1 of 6 animals remaining tumor-free at day 111 (Fig. 5D). Tumor response results are summarized in Table 3.

The antitumor activity of LM against NCI-H526 xenografts when used in combination with carboplatin and paclitaxel was also assessed (Fig. 6). Treatment with LM alone (8.5 mg/kg qw \times

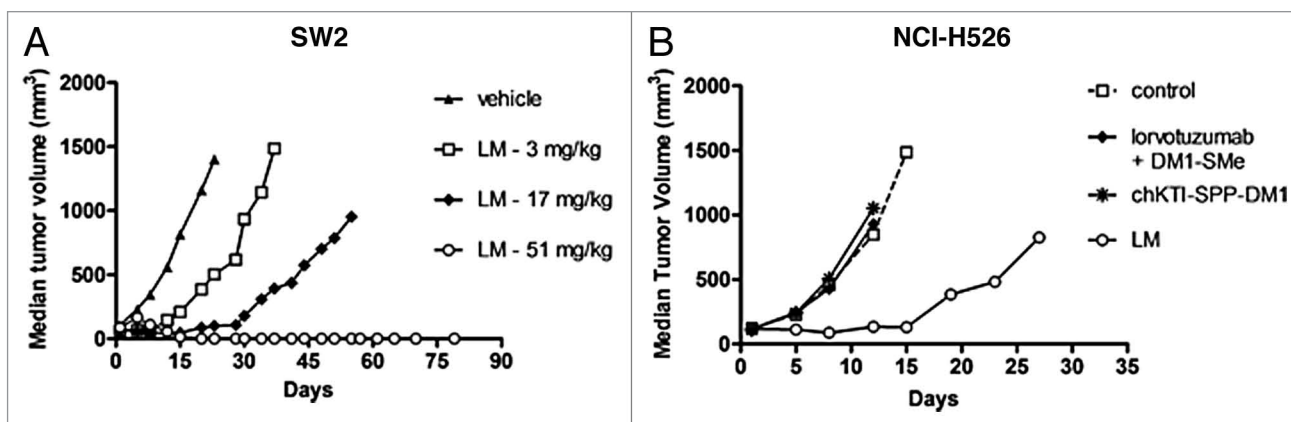


Figure 3. LM has potent single-agent activity in SCLC xenograft models. (A) SCID mice bearing subcutaneous SW2 xenografts were treated with a single intravenous injection of LM at doses of 3, 17, or 51 mg/kg based on antibody dose. Median tumor volumes were plotted vs. time. (B) Athymic nude mice bearing subcutaneous NCI-H526 xenografts were treated with either LM at a dose of 8.5 mg/kg, lorvotuzumab (8.5 mg/kg) and unconjugated DM1-SMe (150 µg/kg, equivalent to the DM1 dose in LM), or a non-targeting conjugate chKTI-SPP-DM1 at an equivalent DM1 dose (150 µg/kg). All treatments were administered intravenously once weekly for two weeks.

2) was active, with 2 of 6 animals tumor-free at end of study (day 111). Carboplatin (100 mg/kg qd × 1) with paclitaxel (10 mg/kg qw × 3) was highly active, with transient complete tumor regressions in 3 of 5 animals, but with tumor regrowth in all animals. The combination of LM/carboplatin/paclitaxel was highly active, with durable complete tumor regressions in all animals (6 of 6) and all animals remaining tumor-free at end of study.

Discussion

The strong expression of CD56 in a majority of SCLC tumors, along with its uniform distribution and cell membrane localization pattern, suggest that targeting CD56 with a maytansinoid-ADC may be a promising strategy for development of a therapeutic for SCLC. We have demonstrated that the humanized anti-CD56 antibody, lorvotuzumab, binds selectively to CD56-positive SCLC cell lines, and that conjugation of lorvotuzumab with maytansinoid does not alter its binding ability. We have also demonstrated that LM has potent antitumor activity both in vitro and in vivo against SCLC models. This cell killing activity requires CD56 targeting and depends of the conjugation of lorvotuzumab with the maytansinoid, as treatments with a non-targeting immunoconjugate or with unconjugated lorvotuzumab plus free maytansinoid were inactive.

SCLC is treated almost exclusively with chemotherapy (plus radiotherapy, in limited-stage SCLC), as so few cases are diagnosed with surgically resectable disease (about 5% of patients).^{6,32} The standard-of-care chemotherapy involves combination of a platinum agent with etoposide, and efforts to improve on the activity of this regimen by including a third agent in the combination have been largely unsuccessful. Addition of ifosfamide to platinum/etoposide did show a modest survival benefit in some patients, but with significantly greater hematologic toxicity.^{33,34} The addition of paclitaxel to platinum/etoposide therapy has been evaluated in several trials due to the observation that single-agent paclitaxel showed some

Table 1. (A) LM has potent single-agent activity in SCLC xenograft models. Activity analysis of LM against SW2 xenografts. (B) Conjugation with DM1 and CD56 targeting is required for antitumor activity.

A			
LM dose (mg/kg)	T/C (%)	Tumor-free (Day 79)	Response
3	29	0 of 6	active
17	6	3 of 6	highly active
51	0	5 of 5	highly active
B			
Treatment	T/C (%)	Tumor-free (Day 111)	Response
lorvotuzumab + DM1-SMe	109	0 of 6	inactive
chKTI-SPP-DM1	124	0 of 6	inactive
LM	16	2 of 6	active

(A) T/C = median tumor volumes of treated (T)/control (C) animals when control tumors reached an average volume of 1000 mm³ (day 17); study ended on day 79. (B) Mice bearing NCI-H526 SCLC xenografts were treated with lorvotuzumab (8.5 mg/kg, qw × 2) plus free DM1-SMe (150 µg/kg, qw × 2); chKTI-SPP-DM1, a non-targeting conjugate at a matched DM1 dose (150 µg/kg, qw × 2); or LM (8.5 mg/kg antibody dose / 150 µg/kg DM1 dose, qw × 2). T/C was calculated when control tumors reached an average volume of 900 mm³ (day 21), study ended on day 111.

clinical benefit in relapsed and refractory SCLC.³⁵ However, this triple combination was found to have increased toxicity and no improvement in overall survival.^{36,37} The majority of chemotherapeutic agents are myelosuppressive, which limits their ability to be combined with other therapies with overlapping toxicity profiles. Addition of agents with hematologic toxicity to a regimen already at its MTD would likely result in intolerable toxicities, require dose reductions, and thus a narrowing of the therapeutic window.

Based on clinical experience in more than 700 patients, maytansinoid-ADCs have been shown to be well tolerated, with favorable toxicity profiles, most notably the lack of clinically

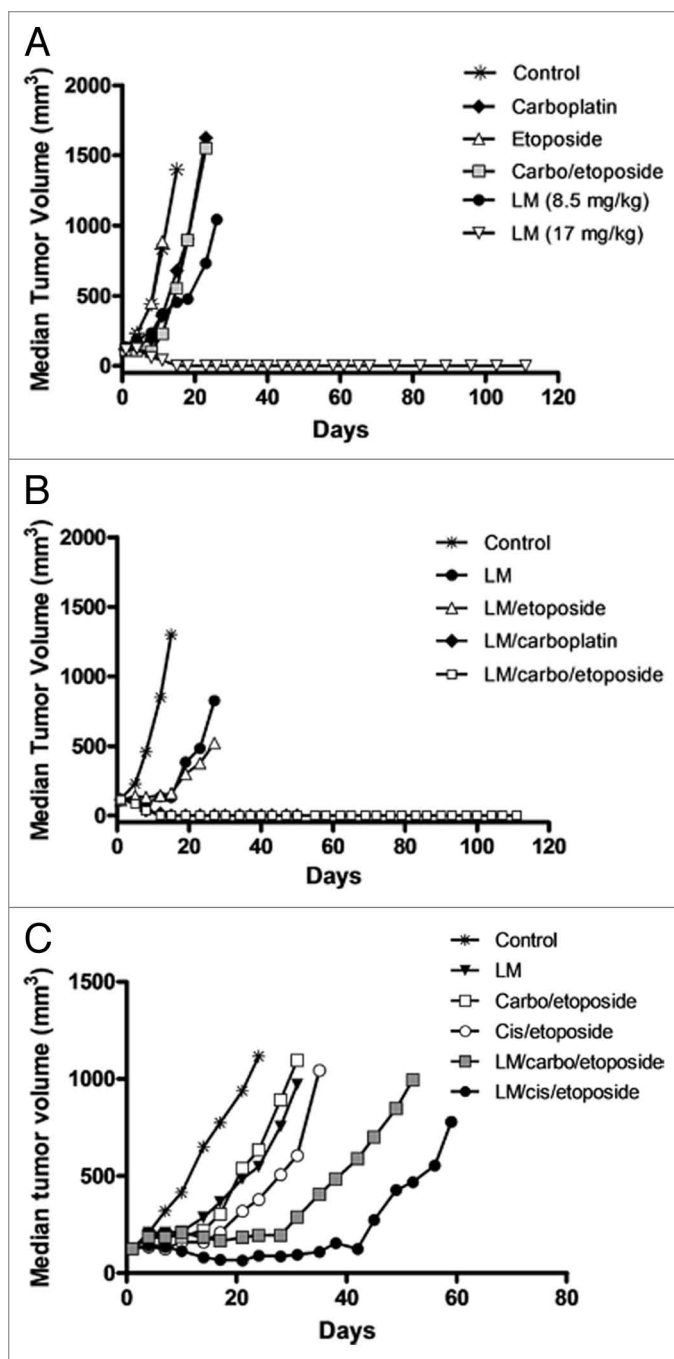


Figure 4. LM is highly active in combination with platinum/etoposide against subcutaneous SCLC xenografts. (A) SCID mice bearing established NCI-H526 xenografts were treated with LM at two dose levels (8.5 or 17 mg/kg, qw \times 2), carboplatin (80 mg/kg qd \times 1), etoposide (3 mg/kg qd \times 5) or the combination of carboplatin/etoposide. (B) In a separate study, combinations of low-dose LM (8.5 mg/kg, qw \times 2) plus carboplatin, etoposide or carboplatin/etoposide at the doses above were evaluated in NCI-H526-bearing mice. (C) Nude mice bearing established NCI-H69 xenografts were treated with LM (18 mg/kg, qw \times 2), carboplatin/etoposide (carboplatin 80 mg/kg qd \times 1/etoposide 5 mg/kg qd \times 5), cisplatin/etoposide (cisplatin 5 mg/kg, qd \times 1/etoposide 8 mg/kg, qd \times 5) or combinations of LM with each platinum/etoposide regimen. Treatments began on Day 10 for NCI-H526 and Day 25 for NCI-H69 tumors. Median tumor volumes were plotted vs. time.

significant myelosuppression.³⁸ This minimal hematologic toxicity may allow combination with a variety of agents of different drug classes, such as the platinum agents, topoisomerase inhibitors and taxanes. In our preclinical evaluations of LM in combination with such agents, we did not observe increased toxicity in terms of body weight loss or clinical observations. The non-overlapping toxicity profile of LM could result in clinically acceptable tolerability profiles when used in combination, thus adding an additional mode of action to the established regimens and offering the possibility for improved outcomes for patients.

Considering the potential tolerability of combination treatments with LM, the preclinical evaluation of such combinations may guide clinical development decisions and dosing paradigms. The combination of LM with standard-of-care platinum/etoposide therapies led to dramatic enhancements in anti-tumor activity in SCLC models, from extended tumor-growth delays to more durable tumor regressions, compared with the chemotherapy regimens alone. The extent of combination activity of LM/carboplatin/etoposide was demonstrated with dose-reduced LM (inactive as monotherapy) resulting in dramatic combination anti-tumor activity relative to chemotherapy alone, even with a LM dose of less than 20% of its MED. The results suggest that LM may be combined successfully with these therapies in the clinic. Furthermore, the remarkable potency of an LM/paclitaxel/carboplatin combination may point to the potential of this regimen to improve upon current standard of care.

The strong activity of LM in combination with these agents may be explained by the activation of different cell death signaling pathways or the sensitization of tumor cells by one agent to the cell killing mechanism of the other agent. Maytansinoid-ADCs have been shown to suppress microtubule dynamic instability, resulting in cell cycle arrest in G2/M and subsequent apoptotic death.³⁹ The platinum/etoposide combinations used as standard-of-care therapy for SCLC have mechanisms of action distinct from maytansinoid-ADCs. Platinum agents act via DNA intra-strand crosslinking, while topoisomerase inhibitors (etoposide) result in double-strand DNA breaks, ultimately leading to apoptosis and necrosis. Etoposide may help overcome one of the pathways of platinum resistance, enhanced DNA repair,⁴⁰ and has been shown to have synergistic activity in combination with cisplatin in cisplatin-resistant cell lines.⁴¹ Notably, although etoposide monotherapy was inactive against NCI-H526 xenografts, it showed enhanced activity when combined with carboplatin vs. carboplatin monotherapy. A similar effect was observed in combinations with LM: the doublet combination with etoposide alone had no benefit over LM monotherapy, yet the triple combination of LM/carboplatin/etoposide was more active than the LM/carboplatin doublet.

Paclitaxel, which like the maytansinoids disrupts microtubule dynamics, has been shown in vitro to have synergistic activity with carboplatin against ovarian cancer cells, and paclitaxel/carboplatin is currently the standard-of-care treatment for ovarian cancer.⁴² The mechanism of paclitaxel/platinum combination synergy is not well-understood. Both paclitaxel

and docetaxel bind to a domain in the tubulin β -subunit on the inside surface of the microtubule, known as the taxane binding site, and act via microtubule-stabilization.⁴³ Maytansine binds to the β -subunit of tubulin dimers, the *vinca*-binding domain, in common with the *vinca* alkaloids, and has been shown to compete with vincristine for binding to tubulin.⁴⁴ While both the maytansinoids and taxanes target tubulin, combination of LM with paclitaxel/carboplatin had surprisingly potent anti-tumor efficacy, which suggests that the binding to different sites on tubulin may contribute to the exquisite combination activity. Synergistic combination activity of other microtubule agents has been reported, for example with combinations of vinorelbine (binds *vinca* domain) and paclitaxel or docetaxel both in vitro and in vivo.⁴³

The preclinical studies reported herein provided rationale for the evaluation of LM in SCLC patients in clinical trials, suggesting that LM has the potential to improve treatment outcomes for SCLC patients. Indeed, LM has been evaluated as monotherapy for the treatment of CD56-positive solid tumors (<http://clinicaltrials.gov/show/NCT00346385>), with a focus on SCLC, Merkel cell carcinoma and ovarian cancer, and is currently being evaluated in combination with etoposide/carboplatin for the treatment of SCLC (<http://clinicaltrials.gov/show/NCT01237678>).

Materials and Methods

Cell lines

NCI-H526 (human SCLC, ATCC CRL-5811), NCI-H69 (human SCLC, ATCC HTB-119) and NCI-N417 (human SCLC, ATCC CRL-5809) were obtained from ATCC (American Type Culture Collection). The SW2 SCLC cell line was obtained from the laboratory of Dr. S. Bernal (Dana-Farber Cancer Institute).⁴⁵ All cell lines were maintained in RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) and 50 μ g/mL gentamicin sulfate in a humidified incubator at 37 °C, 6% CO₂.

Antibodies and immunoconjugates

The CD56-binding humanized antibody lorvotuzumab (huN901), and its murine “parent” antibody, N901, were prepared as described previously.^{14,29} LM (huN901-SPP-DM1) and chKTI-SPP-DM1 (a non-binding control immunoconjugate made from a chimeric human IgG₁ anti-soybean trypsin inhibitor antibody) were synthesized at ImmunoGen, Inc according to published procedures utilizing the heterobifunctional crosslinking agent *N*-succinimidyl-3-(2-pyridyldithio)propionate (SPP).^{11,46} Several lots of LM were used in these studies; all conjugates had acceptable biochemical characteristics including > 97% monomer, <2% free (unconjugated) DM1, with a drug load ranging from 3.3 to 3.6 molecules of DM1 linked per antibody molecule.

Immunohistochemistry (IHC)

CD56 expression was assessed on three formalin-fixed paraffin-embedded (FFPE) SCLC tumor microarrays containing a total of 117 SCLC tumor samples: Biomax LC10010, Biomax LC802 (US Biomax Inc) and Cybridi CS04–11–006 (Cybridi Inc). FFPE cell pellets were prepared from cell culture suspensions

Table 2. LM is highly active in combination with platinum/etoposide against subcutaneous SCLC xenografts.

A			
Treatment	T/C (%)	Tumor-free (Day 110)	Response
LM, 8.5 mg/kg	33	1 of 6	active
LM, 17 mg/kg	0	4 of 6	highly active
carboplatin	49	0 of 6	inactive
etoposide	64	0 of 6	inactive
carboplatin/etoposide	40	0 of 6	minimal activity
B			
Treatment	T/C (%)	Tumor-free (Day 110)	Response
LM	16	2 of 6	active
LM/etoposide	17	0 of 6	active
LM/carboplatin	2	3 of 6	highly active
LM/carboplatin/etoposide	0	5 of 6	highly active
C			
Treatment	T/C (%)	Partial regressions	Response
LM	50	1 of 8	inactive
carboplatin/etoposide	57	0 of 8	inactive
cisplatin/etoposide	34	0 of 8	active
LM/carboplatin/etoposide	19	2 of 8	active
LM/cisplatin/etoposide	7	4 of 8	highly active

(A) SCID mice bearing established NCI-H526 xenografts were treated with LM at two dose levels (8.5 or 17 mg/kg, qw \times 2), carboplatin (80 mg/kg qd \times 1), etoposide (3 mg/kg qd \times 5) or the carboplatin/etoposide. T/C = median tumor volumes of treated (T)/control (C) animals when control tumors reached an average volume of 1300 mm³ (day 15); study ended on day 110. (B) In a separate study, combinations of low-dose LM (8.5 mg/kg, qw \times 2) plus carboplatin, etoposide or carboplatin/etoposide at the doses above were evaluated in NCI-H526-bearing mice. T/C was calculated when control tumors reached an average volume of 900 mm³ (day 12); study ended on day 110. (C) Nude mice bearing established NCI-H69 xenografts were treated with LM (18 mg/kg, qw \times 2), carboplatin/etoposide (carboplatin 80 mg/kg qd \times 1/etoposide 5 mg/kg qd \times 5), cisplatin/etoposide (cisplatin 5 mg/kg, qd \times 1/etoposide 8 mg/kg, qd \times 5) or combinations of LM with each platinum/etoposide regimen. T/C was calculated when control tumors reached an average volume of 1000 mm³ (day 22); study ended on day 70.

of NCI-H526 SCLC and two multiple myeloma cell lines OPM2 and NCI-H929, with CD56 expression levels quantified at the time of cell harvest using a flow cytometry based fluorescence quantitation method utilizing phycoerythrin (PE). NCI-H526, OPM2 and NCI-H929 cells were incubated at 4 °C for about 1 h with a saturating concentration of lorvotuzumab-PE, washed and fixed with 1% formaldehyde (v/v) in phosphate-buffered saline (PBS), and the amount of bound lorvotuzumab-PE was measured by flow cytometry. Anti-CD56 antibodies bound per cell (ABC) values were determined for each test sample by using mean fluorescence value and the PE calibration curve

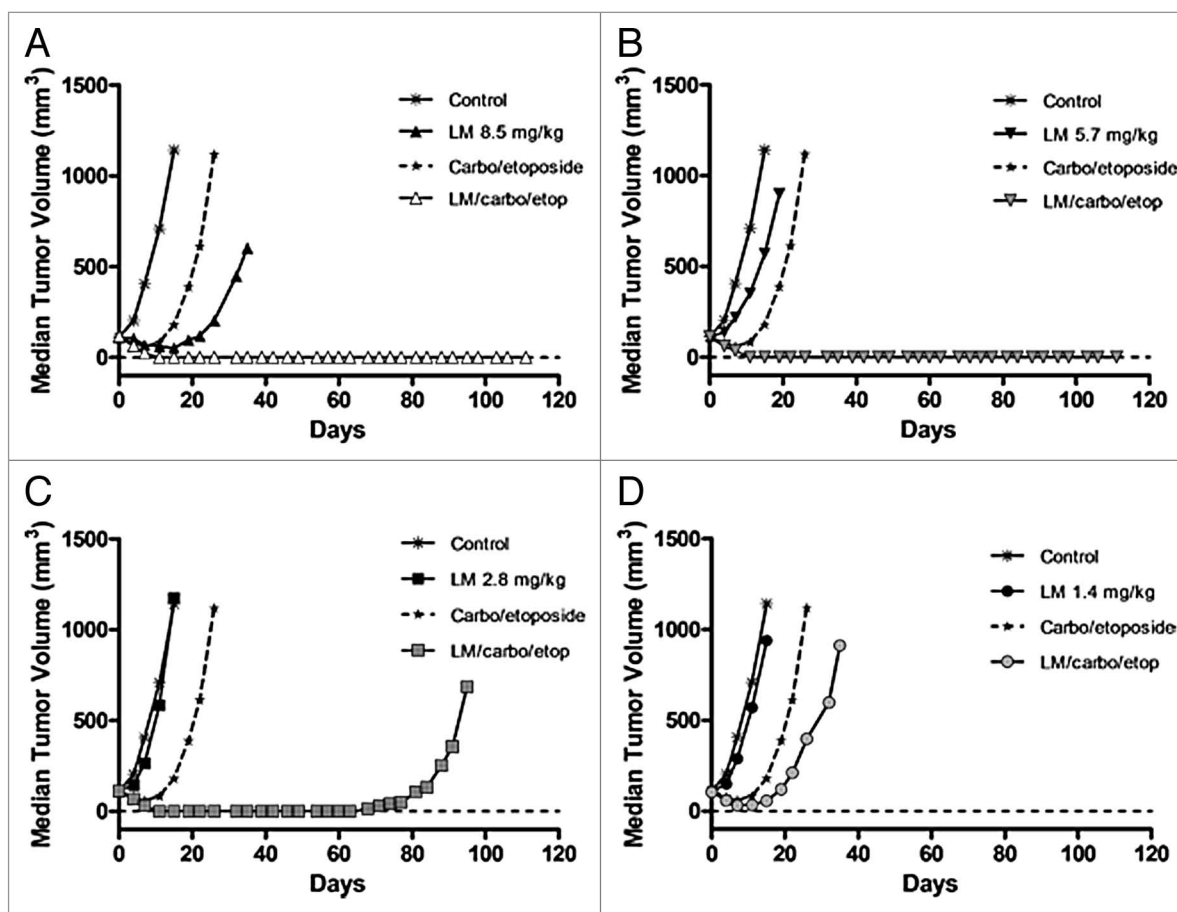


Figure 5. Treatment with LM in combination with carboplatin/etoposide is superior to chemotherapy alone against subcutaneous NCI-H526 SCLC xenografts. SCID mice bearing established NCI-H526 xenografts were treated with single-agent LM at the MED of 8.5 mg/kg (A), and at reduced doses of 5.7 mg/kg (B), 2.8 mg/kg (C) 1.4 mg/kg (D) all at the qw × 2 schedule; carboplatin, 100 mg/kg qd × 1 plus etoposide, 3 mg/kg qd × 5; or the combination of LM with carboplatin/etoposide at the same doses and schedules. Treatments began on Day 9. Median tumor volumes are plotted vs. time.

generated with the Quantibrite™ beads (BD Biosciences, cat. # 340495).

Arrays and cell pellet sections were stained for CD56 using an automated IHC staining protocol with the Leica Bond RX automated staining system (Leica Biosystems) with heat-induced antigen retrieval for 20 min and staining with Novocastra anti-CD56 antibody clone 1B6 (Leica Biosystems, cat. # CD56-1B6) at a concentration of 0.22 µg/mL or isotype control murine IgG1 clone 2T8-2F5 (Beckman Coulter, Cat. # 6602872) at a concentration of 0.22 µg/mL.

All tissue sections were evaluated by a board-certified pathologist to confirm the anatomic site, tumor type, and integrity of the tissue. Samples of poor quality (e.g., necrotic, crushed) and tumor samples that did not contain invasive tumor were excluded. The immunoreactivity of the isotype control samples were first evaluated, followed by test samples. For each tumor tissue or cell pellet evaluated, a description of the staining intensity and respective proportion of tumor cells stained was scored by a board-certified pathologist in terms of intensity and uniformity: staining intensity was scored on a scale of 0–3, 0 = no stain, 1 = weak, 2 = moderate, 3 = strong staining; staining

uniformity (% of cells staining) was scored as homogeneous (>75%), heterogeneous (25–75%) and focal (<25%).

Binding studies

Binding of unmodified antibodies and LM to SW2 SCLC cells was evaluated by an indirect immunofluorescence assay using flow cytometry. Cells (2×10^5 per well) were plated in a round-bottomed 96-well plate and incubated at 4 °C for 1 h with serial dilutions of lorvotuzumab, N901, or LM in 0.2 mL of α-MEM supplemented with 2% (v/v) normal goat serum (Sigma-Aldrich, cat. #G9023). Each sample was assayed in triplicate. Control wells lacked antibody or conjugate. Cells were then washed with 0.2 mL cold (4 °C) medium and stained with fluorescein-labeled goat anti-mouse or anti-human immunoglobulin G (IgG) antibody (Jackson ImmunoResearch, cat. #115-095-164 and #109-095-098) for 1 h at 4 °C. The cells were washed again with medium, fixed in 1% (v/v) formaldehyde in PBS, and analyzed using a FACScan flow cytometer (BD Biosciences).

Cell viability assay

Sensitivity of NCI-H526 and NCI-H69 SCLC cells to LM was evaluated using a cell viability assay utilizing WST-8 (Dojindo Molecular Technologies, Inc, cat. # CCK-8). Briefly,

Table 3. Treatment with LM in combination with carboplatin/etoposide is superior to chemotherapy alone against subcutaneous NCI-H526 SCLC xenografts.

Treatment	LM dose (mg/kg)	T/C (%)	CR	Tumor-free (day 111)	Response
LM single agent	8.5	5	3 of 6	3 of 6	highly active
	5.7	50	0 of 6	0 of 6	inactive
	2.8	103	0 of 6	0 of 6	inactive
	1.4	82	0 of 6	0 of 6	inactive
Carboplatin/etoposide	-	16	0 of 6	0 of 6	active
LM/carboplatin/etoposide combination	8.5	0	5 of 6	5 of 6	highly active
	5.7	0	5 of 6	4 of 6	highly active
	2.8	0	5 of 6	3 of 6	highly active
	1.4	5	1 of 6	1 of 6	highly active

SCID mice bearing established NCI-H526 xenografts were treated with: LM (qw × 2) at doses of 8.5 mg/kg (MED) and at reduced doses of 5.7, 2.8 and 1.4 mg/kg; carboplatin (100 mg/kg qd × 1)/ etoposide (3 mg/kg qd × 5), and combinations of LM and carboplatin/etoposide. T/C = median tumor volumes of treated (T)/control (C) animals when control tumors reached an average volume of 1000 mm³ (day 15); study ended on day 111.

cells were cultured in 96-well plates and treated with serial dilutions of LM with or without excess (1 μM) lorvotuzumab at 37 °C for 8–9 d. After treatments, 20 μL of WST-8 was added to each well and cells were further incubated at 37 °C for 3–4 h. Samples were run in triplicate. Absorbance (450 nm and 650 nm) was measured using a SpectraMax M2 plate reader (Molecular Devices), and EC₅₀ values were generated with GraphPad Prism using a 4-parameter nonlinear regression curve fit (GraphPad Software Inc).

Animal studies

Animal studies were performed in accordance with protocols approved by the Institutional Animal Care and Use Committees (IACUC) of ImmunoGen Inc or Piedmont Research Center.

Human xenograft tumor models in mice

SW2 and NCI-H526 SCLC xenograft tumors were initiated in CB.17 SCID mice (Charles River Laboratories) and NCI-H69 SCLC xenografts were initiated in athymic nude mice (*nu/nu*, Harlan Laboratories). Six-week old female mice received 1 × 10⁷ tumor cells via subcutaneous injection in the flank. Treatments were initiated at average tumor volumes of 90–130 mm³. Tumor size was measured by caliper twice weekly, and volume was calculated using the one of the following formulas:

$$\text{Volume (mm}^3\text{)} = l \times w \times h \times 0.5,$$

$$\text{Volume (mm}^3\text{)} = (w^2 \times l) \times 0.5,$$

where *l* = length, *w* = width and *h* = height of tumor in mm.

Tumor-bearing mice were randomly assigned to treatment groups (5–8 animals per group) based on tumor volume, and treated with PBS (control), lorvotuzumab, DM1-SMe (a mixed disulfide of DM1 with thiomethane to block the free thiol of DM1),⁴⁶ LM or chK₁Ti-SPP-DM1 via intravenous injection at described doses and schedules. Doses of the immunoconjugate are expressed in terms of mg of antibody per kg of body weight (DM1 dose is about 1.5–2.0% of the weight of the ADC). Chemotherapeutics were administered at described doses and schedules, alone or in combination with LM. Cisplatin (Platinol-AQ, Bedford Laboratories) was administered intravenously at a concentration of 0.5 mg/mL in 0.9% (w/v) NaCl. Carboplatin (Paraplatin, Sicor Pharmaceuticals) was

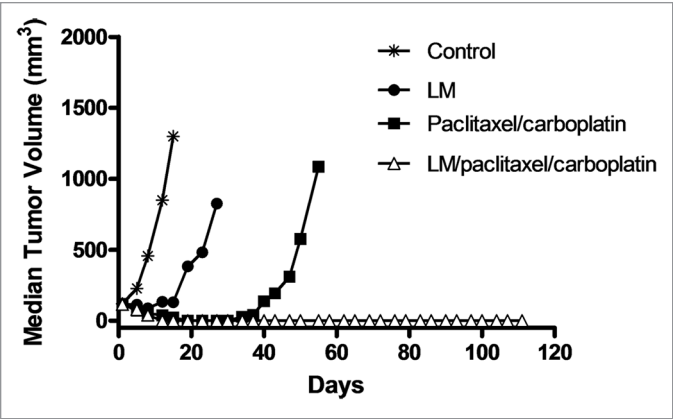


Figure 6. LM is highly active in combination with paclitaxel/carboplatin. SCID mice bearing established NCI-H526 xenografts were treated with LM (8.5 mg/kg, qw × 2), paclitaxel (10 mg/kg, qw × 3) plus carboplatin (100 mg/kg, qd × 1) or the combination of LM/paclitaxel/carboplatin. Treatments began on Day 10. Median tumor volumes are plotted vs. time.

administered intraperitoneally as supplied (10 mg/mL). Etoposide (VePesid, Bristol-Meyers Squibb) was diluted to a concentration of 0.8 mg/mL in 5% (w/v) dextrose in de-ionized water and administered intraperitoneally, or etoposide phosphate (Etopophos, Bristol-Myers Squibb) was reconstituted with sterile water for injection according to package insert, and then further diluted in 0.9% (w/v) NaCl to a concentration of 1 mg/mL immediately prior to intraperitoneal injection. Paclitaxel (Taxol, Bristol-Myers Squibb) was diluted to a concentration of 1.2 mg/mL in 0.9% (w/v) NaCl immediately prior to intravenous injection.

Treatment response criteria

Tumor growth inhibition (T/C), tumor growth delay (T-C) and log cell kill (LCK) were determined according to Bissery et al.⁴⁷ According to NCI standards, tumor growth inhibition (T/C) of ≤42% is the minimum level of anti-tumor activity and a T/C < 10% is considered a high anti-tumor activity level.⁴⁷ A partial tumor regression (PR) was defined as a reduction

in tumor volume by 50% or greater while a complete tumor regression (CR) was scored when no palpable tumor could be detected.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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